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The three-dimensional structure of retinol-binding protein

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The complex of retinol with its carrier protein, retinol-binding protein (RBP) has been crystallized and its three-dimensional structure determined using X-ray crystallography. Its most striking feature is an eight-stranded up-and-down β barrel core that completely encapsulates the retinol molecule. The retinol molecule lies along the axis of the barrel with the β-ionone ring innermost and the tip of the isoprene tail close to the surface.

Key words: vitamin A/retinol/protein-lipid interactions/transport/protein structure

Introduction

Interactions between proteins and lipids are of several types. Integral and transmembrane proteins are wholly or partly embedded in the lipid matrix of a membrane. Another group as exemplified by plasma lipoproteins interacts with the lipid surface to form a coat of protein shielding the lipid core. A third group specifically binds lipid molecules thereby rendering them soluble in an aqueous milieu. This is the method used to transport lipid-soluble vitamin A alcohol (retinol) from the liver to the target cells.

Apart from its role in vision, vitamin A is an essential nutrient and is needed for the proper maintenance of epithelial functions (Olson, 1969; Moore, 1972). However, its molecular mode of action outside the visual process is unknown. Newly synthesized retinol-binding protein (RBP) obtains one molecule of retinol in the endoplasmic reticulum of liver hepatocytes (for a recent review on RBP, see Rask *et al.*, 1980a). This protein is then secreted into the plasma where it occurs in complex with thyroxine-binding prealbumin. RBP, free or in complex with prealbumin, is recognized by a cell surface receptor which transfers the vitamin to the cell. This event affects the conformation of RBP in such a way that its interaction with prealbumin is abolished. The low mol. wt. apo-RBP is filtered through the glomeruli and degraded in the proximal tubuli of the kidney. Thus, each RBP molecule transports just a single retinol molecule before it is degraded.

Human RBP has been sequenced and is composed of 182 amino acid residues (Rask *et al.*, 1979). The complex containing all *trans*-retinol has recently been crystallized by Newcomer *et al.* (1984b) and we are now able to describe its tertiary structure.

Results and Discussion

RBP consists of a single, globular domain ~40 Å in diameter, made up of an N-terminal coil, a β-sheet core, an α-helix and a C-terminal coil (Figures 1 and 2). Its most striking feature is an eight-stranded up-and-down β-barrel core. These anti-parallel strands (A – H) have a +1, +1, +1, +1, +1, +1, +1 topology (Richardson, 1981), wrapped on the surface of a flattened cone such that part of the first strand A is flanked by the last strand H. The loops connecting the strands are all short and for the most part consist of just 2 – 3 residues. The barrel is closed at one end and open to solvent at the opposite end. The retinol molecule lies inside the barrel, pointing along the barrel axis with the β-ionone ring innermost.

When viewed from the flat side of the cone, the barrel appears as two orthogonal β-sheets with the retinol sandwiched in between. The top sheet in Figure 2 consists of strands ABCDEF, and the bottom sheet of the five strands BAHGF, so that A, B and F contribute to both sheets. The main chain hydrogen bond pattern in the core is not entirely regular, particularly the loop 26 – 41. The irregularity is due in part to a severe twist in the strands at residues 27, 41 and 102 where the shared strands cross over. Chothia and Janin (1982) have pointed out that in orthogonal stacked sheets two diagonal corners are closed by shared strands (in this case at residues 40 and 102) while at the other two diagonals the sheets are splayed apart. In addition, most of the inter-sheet contacts occur across the closed corner diagonal. In the RBP core one splayed corner is the entrance to the retinol binding site, and the other corner is very effectively closed by a salt link Lys-17 to Asp-79 on the protein surface, and then an inner ring of five phenylalanine rings (residues 15, 20, 45, 77 and 86) around the side chain of Met-53. The closed corner diagonal

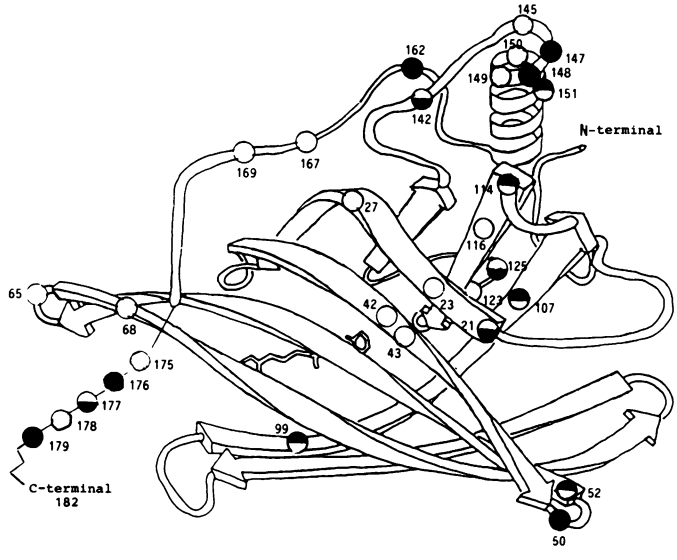

Fig. 1. Retinol-RBP schematic drawing, together with mutations in the human, rat and rabbit sequences. The symbol ◓ represents a change in human sequence from the consensus sequence, ◒ in the rabbit, ○ in rat and ● represents all three differing.

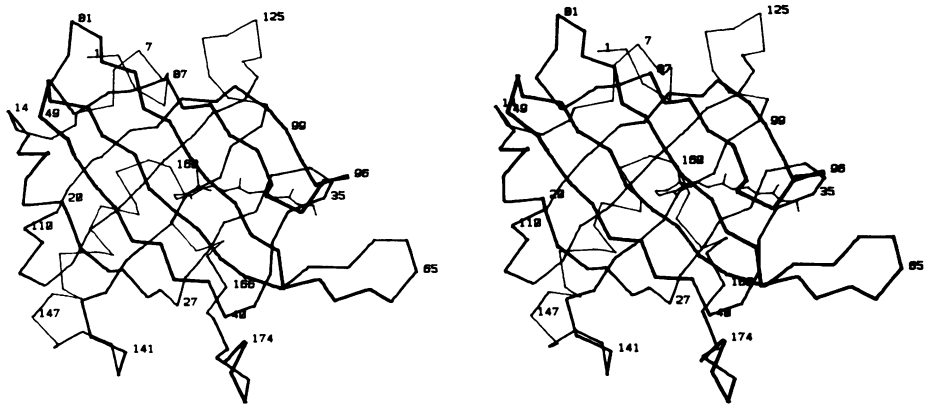

Fig. 2. RBP C α stereo-plot of residues 1 – 175 together with the retinol molecule. The view is chosen to show the orthogonal β-sheets. To enhance the three-dimensional effect, the drawing is made such that the bonds closer to the viewer are drawn thicker.

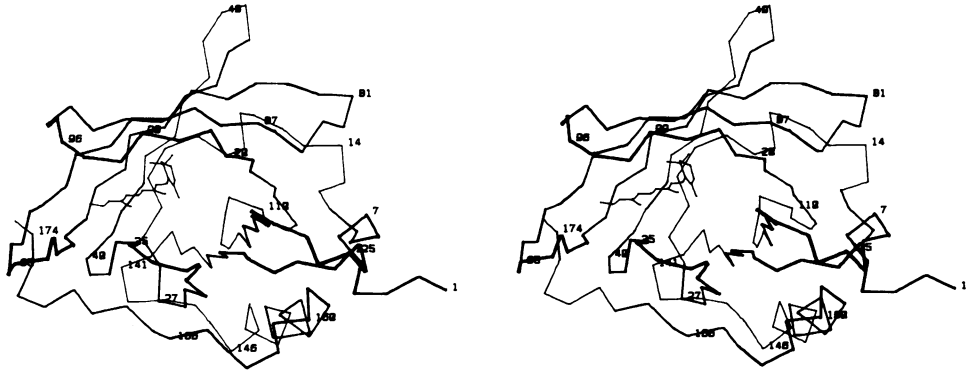

Fig. 3. RBP C α stereo-plot of residues 1 – 175 together with the retinol molecule. The view is into the retinol binding barrel.

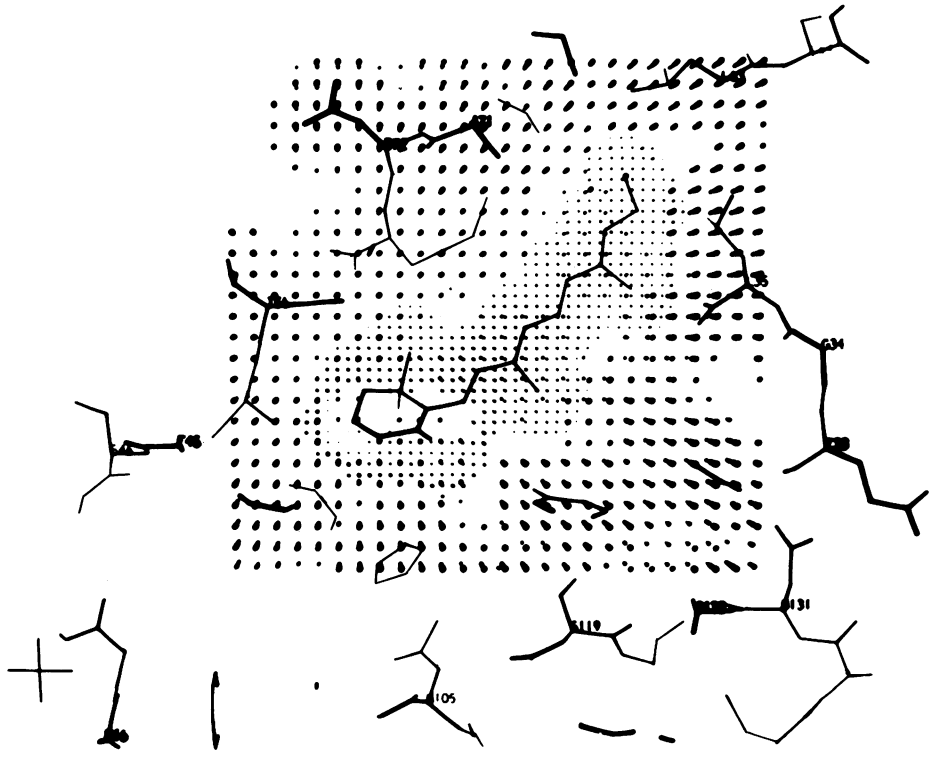

Fig. 4. Retinol binding site with van der Waals surfaces drawn for both retinol and protein to highlight their complementarity. The retinol surface is made up of closely spaced dots, and the protein of widely spaced dots.

contacts are dominated by the retinol molecule which bisects this line. Only one large loop occurs between adjacent strands (G and H) and this is bridged by the disulphide between 120 and 129. The topology of the retinol binding core is rare; the only other eight-stranded anti-parallel barrel known is catalase (Murthy *et al.*, 1981), which has much larger loops connecting the strands, and has a break in the β structure between the fourth and fifth strand.

A three turn α-helix from residues 147 to 160 packs on the underside of the lower sheet making side chain contacts to residues of strands G, H and F. The helix ends with residue Cys-160 which forms a disulphide bridge with Cys-4. Residues 161 – 174 cross the surface of the barrel (strands H, A, B, C and D) to form a disulphide bridge between 70 and 174. Residues 176 – 182 have not yet been located and are probably disordered.

A view into the retinol binding site is shown in Figure 3. The residues lining the pocket are, predictably, hydrophobic or uncharged with contributions from the loop between strands A – B (1 residue) and from strands B (3), C (2), D (2), E (1), F (3) and G (3). Residues within 4 Å of the retinol are Leu-35, Phe-36, Ala-43, Phe-45, Ala-57, Val-61, Met-73, Gly-75, Met-88, Leu-97, Gln-98, His-104, Gln-117, Tyr-133 and Phe-135. The β-ionone ring of the retinol lies deepest in the pocket, with the isoprene tail stretching out almost to the surface of the protein. The molecule is totally buried within the protein (Figure 4), with the area accessible to a water molecule essentially zero. Only the very tip of the retinol is non-zero with the value of 1 Å^2 and this could be due to errors in the coordinates. The hydrophobic nature of the retinol binding site is consistent with the observations that RBP may interact with many different retinyl derivatives provided they contain the β-ionone ring and the conjugated double bond system of the isoprene side chain (Hase *et al.*, 1976). One report has shown that retinyl-phosphate monosaccharides may bind to RBP (Peterson *et al.*, 1976) and the three-dimensional structure supports the notion that the phosphate and sugar moieties will be exposed on the surface of the protein. We are presently carrying out detailed model building studies to predict accurately which retinol analogues should bind to the protein.

With the exception of the single Met-88 of the E strand, the retinol binding pocket of RBP is made up of residues in the strands BCD and FGH. These two sets of strands, as well as strands A and E, are related by an approximate 2-fold rotation axis along the barrel. Residues 36 – 83 form strands BCD plus a turn, while 96 – 141 fold into FGH plus a turn. These stretches exhibit statistically significant homology and it has been suggested that the RBP gene arose by events involving a partial gene duplication (Rask *et al.*, 1980b). The three-dimensional structure is consistent with this idea, but since both sets of strands contribute to the retinol binding pocket it seems unlikely that the ancestral gene of RBP encoded a retinol-binding protein.

RBP delivers retinol to a cell surface receptor expressed in vitamin A-requiring cells (Rask *et al.*, 1980a). It is not known how the transfer of retinol from its hydrophobic environment in RBP to the receptor is accomplished, but after the delivery RBP has virtually lost its affinity for prealbumin (Peterson, 1971). Such apo-RBP, isolated from urine (Peterson, 1971), does not crystallize under the same conditions as the holoprotein. In the absence of retinol, newly synthesized RBP accumulates in the endoplasmic reticulum of hepatocytes (Rask *et al.*, 1983). These data taken together suggest that RBP has different conformations depending on the binding of its ligand. Indeed, removal of the retinol from our model leaves a large, empty volume in the hydrophobic interior of the molecule (Figure 4). This would be most unusual and we must assume that the sheets collapse, probably triggering other conformational changes.

Although the three-dimensional structure of prealbumin known (Blake *et al.*, 1978), the structure of the RBP – prealbumin complex has not yet been elucidated and the residues involved in the contact are unknown. Such contact areas between proteins tend to be evolutionarily conserved since a replacement mutation in one protein may have to be accompanied by a simultaneous and complementary mutation in the other protein to preserve the interaction. Figure 1 shows that RBP from three species (Sundelin *et al.*, 1984) displays a mutation-free area involving the N-terminal residues, the C-terminal base of the α-helix and the loop region around residue 80. This region is rich in charged residues, e.g., 10 out of the first 20 residues in the sequence are charged, which may explain why RBP and prealbumin dissociate at low ionic strength (Rask *et al.*, 1980a). The identity of the prealbumin binding site may be obtained by sequence analysis of piscine RBP which has a considerably lower mol. wt. than mammalian RBP and does not bind prealbumin (Shidoji and Muto, 1977). It is conceivable that piscine RBP binds retinol and interacts with the cell surface receptor just like mammalian RBP, in which case it may consist of just the retinol binding core of mammalian RBP, residues 20 – 140.

We have recently crystallized one of the two main intracellular vitamin A binding proteins (Newcomer *et al.*, 1984a). Unlike RBP this protein, cRBP, displays a higher degree of specificity in its binding of retinol and, in contrast to RBP, it is unable to bind retinoic acid. Since the amino acid sequences of cRBP and RBP have no homology, the elucidation of the structure of cRBP should prove informative as regards this class of protein – lipid interaction.

Materials and methods

The protein was purified and the complex crystallized as described by Newcomer *et al.* (1984b). The structure was solved with X-ray data collected to 3.1 Å resolution by the method of multiple isomorphous replacement (Blow and Crick, 1959) using two derivatives with anomalous data. All map interpretation and model building were made with computer graphics using the program FRODO (Jones, 1978). The starting model had an agreement R-factor of 0.48 with data to 3.1 Å. It has since been crystallographically refined with diffraction data to 2.0 Å using reciprocal space methods (Sussman *et al.*, 1977; Hendrickson and Konnert, 1980) and manual intervention using computer graphics (Jones, 1982). Refinement is not yet complete but the present R-factor is 0.20 to 3.1 Å resolution and 0.24 to 2.0 Å. Full details will be published at a later stage when the refinement is completed.

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